

WHAT IS CLAIMED IS:

1. A method for detecting a secreted mycobacterial antigen in a biological sample obtained from a mammalian subject without substantial replication of mycobacterial cells in said sample, said method comprising:

5 contacting an antibody that specifically binds to said secreted mycobacterial antigen with said sample or a fraction thereof under conditions such that said antibody specifically binds to said secreted mycobacterial antigen in said sample or fraction thereof, thereby forming a specific complex of said antibody with said secreted mycobacterial antigen,
10 and

 detecting the presence, absence or amount of said specific complex of said antibody with said secreted mycobacterial antigen in said sample or fraction thereof, the presence of said specific complex indicating the presence of said secreted mycobacterial antigen in said sample,
15 wherein after said sample is obtained from said subject until said detecting is completed, mycobacterial cells in said sample do not replicate substantially.

20 2. The method of Claim 1 wherein said secreted mycobacterial antigen is an MPB64 antigen or an MPT64 antigen.

 3. The method of Claim 1 wherein said mammalian subject is a human subject suspected of having tuberculosis.

25 4. The method of Claim 3 wherein said biological sample is a sputum sample.

 5. The method of Claim 4 wherein prior to said contacting, a liquifying agent is added to said sample.

6. The method of Claim 5 wherein said liquifying agent comprises a disulfide bond reducing agent.

5 7. The method of Claim 6 wherein said disulfide bond reducing agent is N-acetyl-L-cysteine and sufficient N-acetyl-L-cysteine is added to said sample to achieve a concentration in said sample that is substantially greater than 0.25% (w/v).

10 8. The method of Claim 7 wherein N-acetyl-L-cysteine is added to said sample to achieve a concentration in said sample of about 2.5% (w/v).

15 9. The method of Claim 1 wherein said antibody that specifically binds to said secreted mycobacterial antigen is affixed to a solid matrix.

20 10. The method of Claim 9 wherein said detecting the presence, absence or amount of said specific complex of said antibody with said secreted mycobacterial antigen comprises using a reagent that produces a color that indicates the presence of said specific complex.

25 11. The method of Claim 1 wherein
prior to said contacting, said sample is treated to separate mycobacterial cells from the remainder of said sample, thereby providing a mycobacterial cell fraction and a remainder fraction of said sample,
said mycobacterial cell fraction is tested for the presence of mycobacterial cells, and

said antibody that specifically binds to said secreted mycobacterial antigen is affixed to a solid matrix and is contacted with said remainder fraction of said sample.

5 12. The method of Claim 11 wherein said mycobacterial cell fraction is prepared by passing said sample through a filter that retains mycobacterial cells and does not retain MPB64 antigen or MPT64 antigen, whereby the resulting retentate on said filter becomes the mycobacterial cell fraction and the resulting filtrate becomes the remainder fraction.

10 13. A method for detecting an infection of a mammalian subject by a mycobacterial species that produces a secreted mycobacterial antigen by testing a biological sample obtained from said subject for said secreted mycobacterial antigen without substantial replication of mycobacterial cells in said sample, said
15 method comprising:

 contacting an antibody that specifically binds to said secreted mycobacterial antigen with said sample or a fraction thereof under conditions such that said antibody specifically binds to said secreted mycobacterial antigen in said sample or fraction thereof, thereby forming a
20 specific complex of said antibody with said secreted mycobacterial antigen, and

 detecting the presence, absence or amount of said specific complex of said antibody with said secreted mycobacterial antigen in said sample or fraction thereof, the presence of said specific complex indicating infection
25 of said subject by said mycobacterial species,
 wherein after said sample is obtained from said subject until said detecting is completed, mycobacterial cells in said sample do not replicate substantially.

14. The method of Claim 13 wherein said secreted mycobacterial antigen is an MPB64 antigen or an MPT64 antigen.

15. A method of processing a biological sample obtained from a mammalian subject for detecting MPB64 antigen or MPT64 antigen in said sample comprising

adding a liquification agent to said sample prior to said detecting, and after said sample is obtained from said subject until said detecting is completed, maintaining said sample at a substantially neutral pH and under conditions such that mycobacterial cells in said sample do not replicate substantially.

16. The method of Claim 15 wherein said liquifying agent comprises N-acetyl-L-cysteine in an amount sufficient to achieve a concentration in said sample that is substantially greater than 0.25% (w/v).

17. The method of Claim 16 wherein said liquifying agent comprises N-acetyl-L-cysteine in an amount sufficient to achieve a concentration in said sample of about 2.5% (w/v).

18. The method of Claim 15 wherein said liquifying agent comprises a DNase that is capable of cleaving DNA in said biological sample.

19. The method of Claim 15 further comprising adding a decontamination agent to said sample prior to said detecting.

20. The method of Claim 19 wherein said decontaminating agent comprises polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin in amounts sufficient to achieve concentrations in said sample of at least about 50 U/ml, 5 micrograms/ml, 20 micrograms/ml, 5 micrograms/ml and 10
5 micrograms/ml, respectively.

21. The method of Claim 20 wherein said decontaminating agent comprises polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin in amounts sufficient to achieve concentrations in said sample of about
10 500 U/ml, 50 micrograms/ml, 200 micrograms/ml, 50 micrograms/ml and 100 micrograms/ml, respectively.

22. An article of manufacture comprising a container, a liquification agent and packaging material containing said container and said liquification agent,
15 wherein said packaging material includes a label that indicates that said container is to be used for combining said liquification agent with a biological sample obtained from a mammalian subject for detecting MPB64 antigen or MPT64 antigen in said sample or a fraction thereof without substantial replication of mycobacterial cells in said sample.

20 23. The article of manufacture of Claim 22 wherein said liquification agent is N-acetyl-L-cysteine in a solid form.

24. The article of manufacture of Claim 23 wherein said solid form further
25 comprises a decontamination agent.

25. The article of manufacture of Claim 22 wherein said liquification agent is contained in said container so that a biological sample introduced into said container contacts said liquification agent.

5 26. The article of manufacture of Claim 25 wherein said liquification agent is in the form of a coating on a surface inside of said container.

27. The article of manufacture of Claim 22 wherein said container further comprises a first solid support and a second solid support, wherein

10 said first solid support retains mycobacterial cells and does not retain MPB64 antigen or MPT64 antigen,

 said second solid support has affixed thereto a capture antibody that specifically binds to MPB64 antigen or MPT64 antigen, and

 said first solid support and said second solid support are disposed inside

15 said container such that a biological sample introduced into said container contacts said first solid support and said second solid support.

28. The article of manufacture of Claim 27 wherein said first solid support comprises a first porous matrix and said second solid support comprises a second

20 porous matrix, wherein said first porous matrix and said second porous matrix are disposed so that when a biological sample is introduced into said container,

 said biological sample passes through said first porous matrix, whereby mycobacterial cells in said sample are retained by said first porous matrix, and thereafter

25 said biological sample passes through said second porous matrix, whereby MPB64 antigen or MPT64 antigen in said sample is specifically bound to said capture antibody on said second porous matrix.

29. The article of manufacture of Claim 28 wherein said first solid matrix is removable from said container after said biological sample passes through said first porous matrix such that mycobacterial cells retained by said first porous matrix are removed from said container on said first porous matrix.

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30. The article of manufacture of Claim 28 further comprising a detection reagent that specifically binds to said MPB64 antigen or MPT64 antigen that is specifically bound to said capture antibody and thereby generates a detectable signal on said second porous matrix.

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31. The article of manufacture of Claim 30 wherein
said container further contains all reagents needed for detecting MPB64 antigen or MPT64 antigen in a biological sample including a reagent that produces a color that indicates the presence of MPB64 antigen or MPT64 antigen
specifically bound to said capture antibody on said second porous matrix, and
said label indicates that said container contains all reagents needed for detecting MPB64 antigen or MPT64 antigen in a biological sample that is introduced into said container.

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